

WEST Search History

DATE: Monday, August 19, 2002

<u>Set Name</u>	<u>Query</u>	<u>Hit Count</u>	<u>Set Name</u>
side by side			result set
<i>DB=USPT,PGPB,JPAB,EPAB,DWPI; PLUR=YES; OP=OR</i>			
L4	nucleic with (array or microarray or immobiliz\$4) with (scratch\$4 or shav\$4)	1	L4
L3	nucleic with (array or microarray or immobiliz\$4) with rub\$5	3	L3
L2	nucleic with remove with pipet\$4	10	L2
L1	nucleic with transfer\$4 with pipet\$4	36	L1

END OF SEARCH HISTORY

WEST

Generate Collection

Print

L2: Entry 6 of 10

File: USPT

DOCUMENT-IDENTIFIER: US 5681946 A

TITLE: Precipitating polymers

Detailed Description Text (47):

Precipitations of plasmid (e.g. pBR322) can be performed according to the following protocol: Take pBR322 DNA in, for example, 100 . μ l of TE buffer (10 mM Tris-HCl (pH 8.0), 1 mM EDTA (pH 8.0)); add 1/10th volume (i.e. 10 . μ l) of a solution containing magnetic beads at 50 mg/ml and tRNA at 1 mg/ml (as carrier, this may be omitted if the concentration of nucleic acid is greater than about 10-25 . μ g/ml) in 2.5M sodium acetate adjusted to pH 5.2 with acetic acid; mix; add 2.5 volumes (i.e. 250 . μ l) of ethanol; mix; place over a permanent magnet to bring down the precipitate; remove the supernatant; wash the pellet in, for example, 100 . μ l of 70% (v/v) ethanol by resuspending with a pipette in the absence of the magnetic field, finally removing the supernatant (wash solution) with a pipette in the presence of the magnetic field; redissolve the pellet in the required volume of TE buffer.

Detailed Description Text (50):

Precipitation of pBR322 plasmid DNA with deproteinization can be performed according to the following protocol: Take, for example, pBR322 DNA in 20 . μ l of TE buffer (10 mM Tris-HCl (pH 8.0), 1 mM EDTA (pH 8.0)) containing protein (e.g. a 1/4 dilution of Rainbow Markers.TM. (Amersham International)); add 1/10th volume (i.e. 2 . μ l) of a solution containing magnetic beads at 50 mg/ml and tRNA at 1 mg/ml (as carrier, this may be omitted if the concentration of nucleic acid is greater than about 10-25 . μ g/ml) in 2.5M sodium acetate adjusted to pH 5.2 with acetic acid; mix; add 2.5 volumes (i.e. 50 . μ l) of ethanol; mix; place over a permanent magnet to bring down the precipitate; remove the supernatant; wash the pellet in, for example, 50 . μ l of phenol (or greater than 60% (v/v) phenol in ethanol) by resuspending with a pipette in the absence of the magnetic field, finally removing the supernatant (wash solution) with a pipette in the presence of the magnetic field; redissolve the pellet in the required volume of TE buffer. The yield of DNA falls off with less than 40% (v/v) phenol in ethanol used for protein extraction. No losses are incurred with this additional step of protein extraction compared to a protein-free ethanol precipitation. Ethanol precipitation from a solution heavily contaminated with protein is also seen to be dependent upon the extraction of the contaminating protein by a phenol containing solution (i.e. the DNA cannot be redissolved from the beads if protein extraction has not been performed). The successful extraction of the protein into the phenolic layer by this procedure can clearly be seen when using coloured proteins. The above procedure works equally well for human genomic DNA and for RNA.

Detailed Description Text (56):

DNA preparations can be performed on, for example, 1 ml samples of M13mp8 bacteriophage in 2xTY broth (precleared of bacteria by centrifugation) according to the following protocol: Add 0.4 volumes (i.e. 400 . μ l) of 2 mg/ml magnetic beads in 20% (w/v) PEG, 2.5M NaCl; mix; bring down magnetic beads and precipitate using a permanent magnet; redissolve magnetic bead pellet in 1/5th volume (i.e. 200 . μ l) of TE buffer; extract with an equal volume (i.e. 200 . μ l) of phenol; remove aqueous (top) layer; add 1/10th volume (i.e. 20 . μ l) of a solution containing magnetic beads at 50 mg/ml and tRNA at 1 mg/ml (as carrier, this may be omitted if the concentration of nucleic acid is greater than about 10-25 . μ g/ml) in 2.5M sodium acetate adjusted to pH 5.2 with acetic acid; mix; add 2.5 volumes (i.e. 500 . μ l) of ethanol; mix; place over a permanent magnet to bring down the precipitate; remove the supernatant; wash the pellet in, for example, 100 . μ l of 70% (v/v) ethanol by resuspending with a pipette in the absence of the magnetic field, finally removing the supernatant (wash solution) with a pipette in the presence of the magnetic field; redissolve DNA in the required volume of TE buffer.

Detailed Description Text (61):

pUC19 DNA can be extracted from E. coli MC1061 cells by the following protocol: Take, for example, 250 .mu.l of bacterial culture; add 1/5th volume (i.e. 50 .mu.l) of 1.2M NaOH, 1.2% (w/v) SDS; mix; incubate 2 minutes at room temperature; now add 3/5th volume (i.e. 150 .mu.l) of 10 mg/ml magnetic beads in 3M potassium acetate adjusted to pH 4.8 with acetic acid; mix; bring down precipitated material with a permanent magnet and keep supernatant; isopropanol precipitate the supernatant as follows: add 1/10th supernatant volume (i.e. 45 .mu.l) of a solution containing magnetic beads at 50 mg/ml and tRNA at 1 mg/ml (as carrier, this may be omitted if the concentration of nucleic acid is greater than about 10-25 .mu.g/ml) in 2.5M sodium acetate adjusted to pH 5.2 with acetic acid; mix; add 0.6 supernatant volumes (i.e. 270 .mu.l) of isopropanol; mix; place over a permanent magnet to bring down the precipitate; remove the supernatant; wash the pellet in, for example, 100 .mu.l of 70% (v/v) ethanol by resuspending with a pipette in the absence of the magnetic field, finally removing the supernatant (wash solution) with a pipette in the presence of the magnetic field; redissolve DNA in the required volume of TE buffer. Preparations can be incubated with 10 .mu.g/ml ribonuclease A for 10 minutes at 37.degree. C. before analysis.

Detailed Description Text (63):

pUC19 DNA can also be extracted from E. coli MC1061 cells by the following protocol: Take, for example, 500 .mu.l of bacterial culture; add 1 volume (i.e. 500 .mu.l) of a solution containing magnetic beads at 5 mg/ml in 0.2M sodium acetate (adjusted to pH 5.2 with acetic acid) dissolved in ethanol; mix; bring down precipitated bacteria with a permanent magnet; remove supernatant and discard; redissolve bacterial pellet in, for example, 300 .mu.l of 0.2M NaOH, 0.2% (w/v) SDS; mix; incubate 2 minutes at room temperature; now add 1/2 volume (i.e. 150 .mu.l) of 3M potassium acetate adjusted to pH 4.8 with acetic acid; mix; bring down precipitated material with a permanent magnet and keep supernatant; isopropanol precipitate the supernatant as follows: add 1/10th supernatant volume (i.e. 45 .mu.l) of a solution containing magnetic beads at 50 mg/ml and tRNA at 1 mg/ml (as carrier, this may be omitted if the concentration of nucleic acid is greater than about 10-25 .mu.g/ml) in 2.5M sodium acetate adjusted to pH 5.2 with acetic acid; mix; add 0.6 supernatant volumes (i.e. 270 .mu.l) of isopropanol; mix; place over a permanent magnet to bring down the precipitate; remove the supernatant; wash the pellet in, for example, 100 .mu.l of 70% (v/v) ethanol by resuspending with a pipette in the absence of the magnetic field, finally removing the supernatant (wash solution) with a pipette in the presence of the magnetic field; redissolve DNA in the required volume of TE buffer. Preparations can be incubated with 10 .mu.g/ml ribonuclease A for 10 minutes at 37.degree. C. before analysis.